Detection of chemical engagement of solute carrier proteins by cellular thermal shift assay

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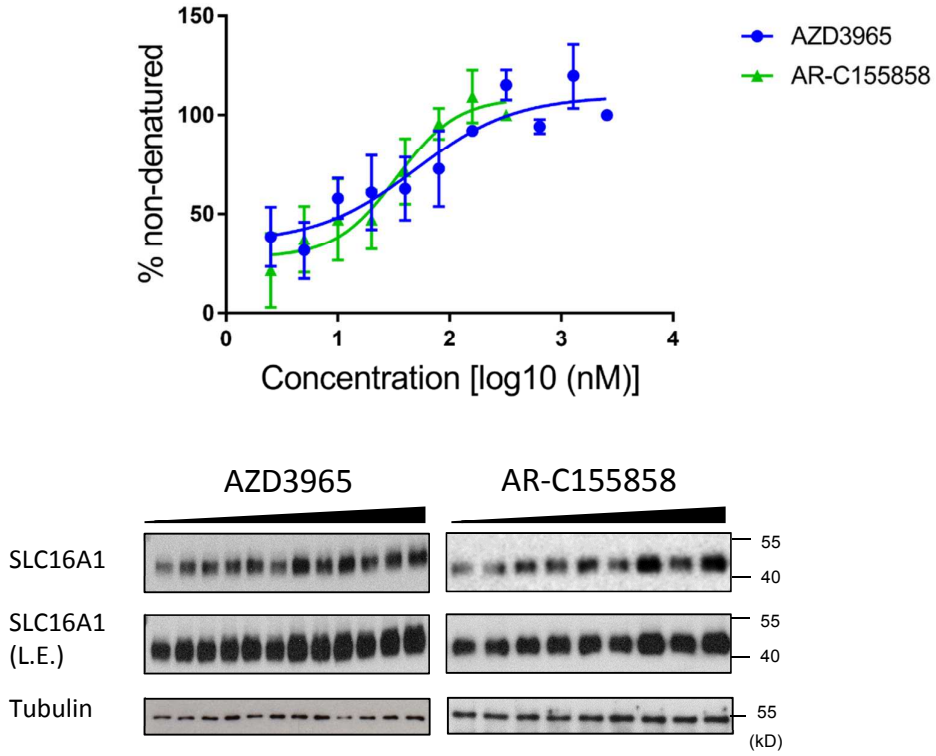
Supplementary methods

Vectors The following plasmids were cloned for this study: EGFP-FLAG/pCI, SLC1A2/pCI and SLC1A2-FLAG/pCI. All of these constructs are based on the mEGFP-Hras plasmid (Addgene #18662), from which HRAS was removed by double digestion with Xho1 and Not1 (New England BioLabs). Synthesized oligos coding for the FLAG sequence with enzyme recognition sites were annealed in T4 DNA ligase buffer containing T4 polynucleotide kinase (New England BioLabs), and sequentially ligated into the linearized mEGFP/pCI plasmid, resulting in the mEGFP-FLAG/pCI plasmid. For SLC1A2-FLAG/pCI, the FLAG/pCI fragment was obtained from mEGFP-FLAG/pCI by double digestion with Nhe1 and Xho1 (New England BioLabs), and ligated to an SLC1A2 fragment subcloned from the CMV-hEAAT2 plasmid (Addgene, #32814). The empty pCI fragment was produced from the mEGFP-FLAG/pCI plasmid by digestion with Not1 and Nhe1, and ligated to an hSLC1A2 fragment, resulting in SLC1A2/pCI.

Transient expression HEK293 cells were seeded in a sterile 24-well culture plate at $2x10^5$ cells/well and incubated overnight. Using lipofectamine 2000 (Thermo Fisher Scientific), each well was transfected with 500 ng of each plasmid. After 48 h, cells were washed with PBS and harvested in 300 µl lysis solution (100 mM ammonium sulfate, 400 mM NaCl, 10% glycerol, 0.5% DDM) supplemented with proteinase inhibitor cocktail.

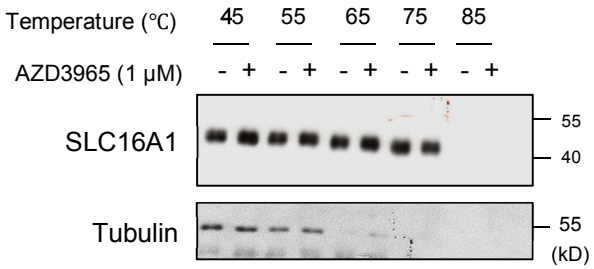
N-glycosidase F (PNGase F) treatment For protein de-glycosylation by PNGase F, cell lysates (3.2 µg protein) were denatured by 1 h incubation at 37 °C in 10 µl 0.5% SDS containing 40 mM dithiothreitol. The samples were mixed with 13 µl 0.5 M sodium phosphate (pH 7.5) containing 1% (v/v) NP-40, and 2 µl PNGase F solution (New England BioLabs). The mixture was incubated in a volume of 25 µl (final protein concentration = 0.13 µg/µl) for 1 h at 37 °C. The reaction was stopped by adding an equal volume of SDS-PAGE loading buffer, and samples were subsequently analyzed by immunoblotting.

Immunocytochemistry HEK293 cells were transfected with SLC1A2-FLAG/pCI as described above and processed 48 h post transfection. All experiments were carried out at room temperature unless indicated otherwise. Following washing with PBS, cells were fixed and permeabilized in PBS containing 4.0 % formalin and 0.1% Triton X-114 for 20 min, and blocked for 30 min in 5% BSA in PBS. Then, cells were incubated with mouse monoclonal anti-FLAG M2 antibody (1:200) in 5% BSA-PBS for 1 h. Following four 5 min washing steps in PBS, cells were incubated with an Alexa Fluor 680-conjugated anti-mouse secondary antibody (1:150, Thermo Fisher Scientific) for 1 h. Cell nuclei were then stained by DAPI, washed four times for 5 min each with PBS, and mounted on microscope slides. A laser scanning microscope (LSM700, Zeiss) was used for imaging.



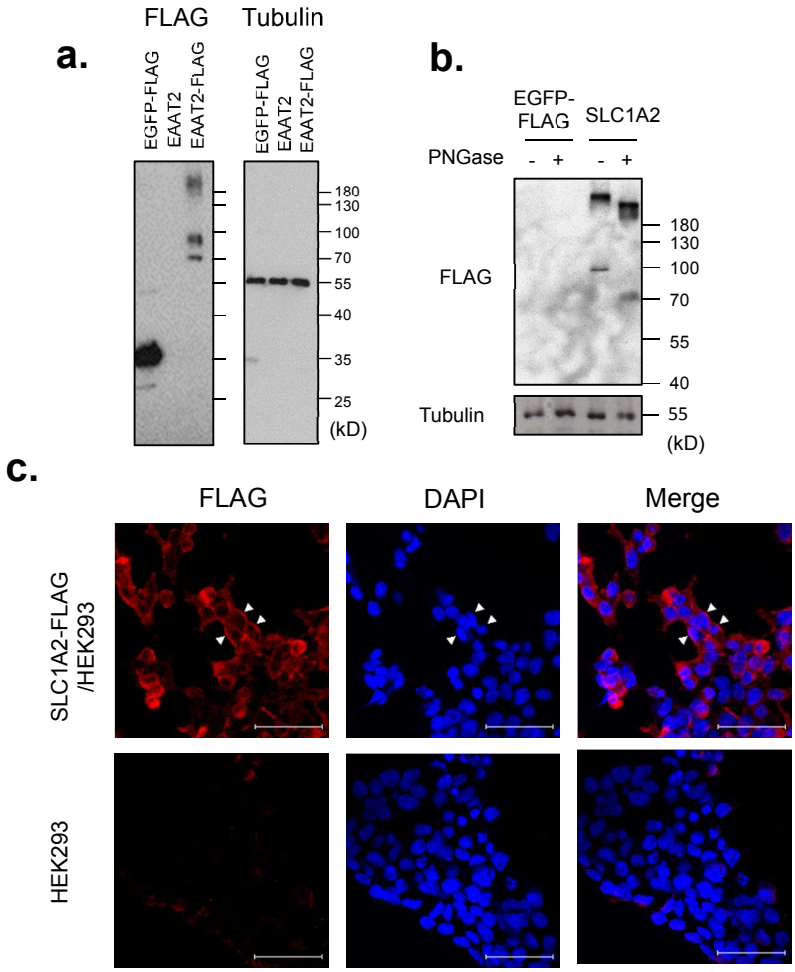
Supplementary figure. 1 Isothermal drug-response fingerprinting for SLC16A1 inhibitors in intact cells.

For isothermal drug response fingerprinting in intact HEK293 cells, cells were cultured and treated with AZD3965 (0, 2.5, 5, 10, 20, 40, 80, 160, 320, 640, 1280, 2560, 5120 nM) and AR-C155858 (0, 2.5, 5, 10, 20, 40, 80, 160, 320 nM) as described in the method section. The cell lysates were heated at 65 ℃ for 6 min and subjected to immunoblot analysis. Abundance of thermostable SLC16A1 was assessed and drug response curves were fitted using GraphPad Prism 7. Data are mean.±S.D., n=3. L.E.; long exposure



Supplementary figure 2. Cell-based CETSA on SLC16A1 in NP-40 containing buffer.

For CETSA in living, intact HEK293 cells, cells were cultured and treated with AZD3965 (1 μM) as described in the method section. Following incubation, the cells were washed with PBS to remove excess drug, and suspended in 500 μl of lysis buffer [50 mM Tris-HCl, 100 mM NaCl, 0.2% (v/v) NP-40, 5% (v/v) glycerol, 1.5 mM MgCl2, 25 mM NaF, 1 mM Na3Cl2, 1 mM Na3VO4, 1 mM PMSF, 1 mM DTT, 0.1% (v/v) protein inhibitor cocktail, 10 μg ml^{-1} TLCK), in the culture plate. The cell lysates were transferred to tubes, snap-frozen in liquid nitrogen, and then placed at room temperature. After about a half of cell lysate was thawed, they were moved onto ice until the entire content was thawed. This freeze-thaw cycle was repeated twice. The cell lysate was then centrifuged at 14,000 x g for 20 min at 4 ℃ , and transferred to 1.5 ml Eppendorf tubes. Heat treatment and immunoblotting were performed as described in the method section.



Supplementary figure 3. Transient expression of SLC1A2 tagged with FLAG in HEK293 cells.

a) The protein expression level of SLC1A2-FLAG was examined in whole cell lysates of HEK293 cells transfected with EGFP-FLAG/pCI (control), SLC1A2/pCI or SLC1A2-FLAG/pCI. b) The expression of SLC1A2 with (+) or without (-) PNGase treatment was analysed in EGFP-FLAG and SLC1A2-FLAG transfected cells. c) The subcellular localization of SLC1A2-FLAG was analysed using immunofluorescence staining with an anti-FLAG antibody. Arrows indicate membrane localized SLC1A2. Staining of HEK293 wild type cells is also shown as a negative control (lower panel). DAPI was used for nuclei staining. Scale bar is 50 µm. Results presented in a-c represent two biological replicates.